Snubnose-like (*snol*), a new spontaneous skeletal mutation on Chromosome 4 in the mouse

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Mutation Symbol: *snol*

Mutation Name: snubnose-like

Strain of origin: C3H/HeJ

Current strain name:C3H/HeJ-*snol*/GrsrJ

Stock #004476 (jaxmice.jax.org)

Phenotype category: craniofacial, skeletal, tail, malocclusion

Abstract

snol is a new autosomal recessive mutation that arose spontaneously in a C3H/HeJ colony at The Jackson Laboratory. The homozygous mutant phenotype includes a short nose, odd face and body shape, and kinked tail. Most mutants also get malocclusion. Both heterozygous and homozygous mutants carry *rd1*, but this is a C3H/HeJ strain characteristic. We used an intercross between a C3H mutant and CAST/Ei to map the *snol* mutation to Chromosome 4. The most likely gene order places the mutation between *D4Mit12* and *D4Mit203* in 92 tested meioses. A short nosed mutation, snubnose (*sno*), maps in this location, but could not be tested for allelism because it is believed to be extinct. The spina bifida occulta reported in *sno* is not seen in *snol*. Mouse mutants demonstrating facial and skeletal defects are important models for a variety of human craniofacial abnormalities. Such mutant animals are valuable tools for the study of the pathology contributing to these abnormalities and can aid in the molecular identification of responsible genes. Among the 67 craniofacial genes and mutations listed in the Mouse Genome Database (MGD 2002) are several mutants exhibiting similar short nosed phenotypes. This mutant is different than other similar mutations because of its chromosomal map location.

Origin and Description

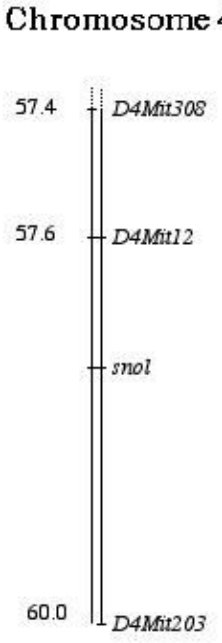
The *snol* mutation is recessive and arose spontaneously in the C3H/HeJ strain of mice in a production colony at the Jackson Laboratory in 1999. Homozygous mutants are recognizable at about 14 days of postnatal development by their odd shaped face. The mutant mice may live to adulthood and breed, though some die by weaning. The *snol* mutation is maintained by mating a homozygote with a heterozygote or a heterozygote with a heterozygote.

Genetic Analyses

snol is inherited as a recessive mutation as shown by traditional linkage cross analysis. No

visible mutants were seen in the F1 generation (0/66) and about 9% of the F2 progeny were mutant (53 *snol*/592 total progeny), much lower than the expected 25%.

For linkage analysis, an intercross was utilized to produce mutant mice. CAST/Ei females were mated to C3H/HeJ-*snol/snol* males. F1 hybrids from this initial cross were then intercrossed to produce the F2 progeny. The F2 progeny were scored visually for phenotype and spleens and tail tips from 46 *snol/snol* animals were collected and stored at -70C for subsequent DNA typing to map the mutation. DNA was extracted by a standard hot sodium and Tris (HotSHOT) procedure (Truett,et al., 2000). PCR reactions with MIT primer pairs (MapPairs, Research Genetics, Huntsville Ala.) were carried out in 10 ul total volume containing 20 ng genomic DNA, by previously described methods (Ward-Bailey et al. 1996). Mutation segregation ruled out Chr X linkage. A genome sweep was begun with markers on Chr 4, because several mutations with similar snubnose phenotypes are located there. Linkage of *snol* was first detected with *D4Mit308*. DNA samples were then typed for three additional Chr 4 markers. The recombination estimates and best gene order are centromere - *D4Mit308* - 1.09+/-1.08 - *D4Mit12* - 1.09+/-1.08 - ***snol*** -2.19+/-1.52 - *D4Mit203*. Gene order and recombination frequencies were calculated with the Map Manager computer program (Manley 1993). The complete Chr 4 linkage data for 46 F2 *snol/snol* mice have been deposited in the Mouse Gemone Database, accession number J:78986. The map position for *snol* is 58.0, according to MGD placement.



Pathology

Tissues for histopathological examination were prepared from both *snol/snol* and control animals. Tissues were removed from animals deeply anesthetized with tribromoethanol (Avertin) and fixed by intracardiac perfusion of Bouin's solution following a flush of saline. After demineralization in 'Bouin', multiple cross sections of all portions of the spinal cord and brain were prepared. Cross and longitudinal sections of lumbar muscles, fore and hindlimb muscles and samples of each of the somatic organs (liver, spleen, pancreas, stomach, small intestine, colon, cecum, lungs, thymus and heart) were also prepared. For light microscopy sections of all tissues were stained with hematoxylin and eosin (H&E). Brain and spinal cord sections were also stained with luxol-fast blue-cresylecht violet (LFB-CV) and Bodian's stain.

Specimens from 2 *snol/snol* and 2 +/? mice were cleared and stained with alizarin red S and alcian blue to demonstrate bone and cartilage (Green, M.C. 1952). No consistent histological lesions were observed.

An ophthalmoscope was used to view the eyes of two 4-month old *snol*/+ mice and one 6-month old *snol/snol* mouse. All had retinal degeneration because of *rd1* from the C3H strain background.

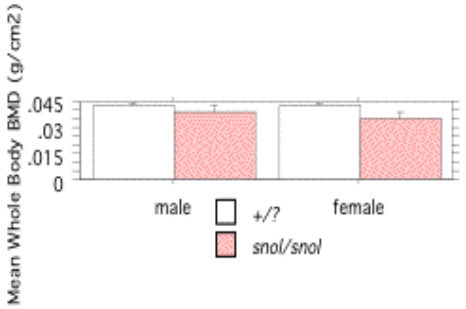
Hearing was assessed by ABR threshold analysis (Zheng et al. 1999). The ABR results showed that two homozygous mutant mice tested at 49 days of age exhibited intermediate hearing loss (about 25 dB above normal) and two +/? littermate control mice had normal hearing.

Graphs

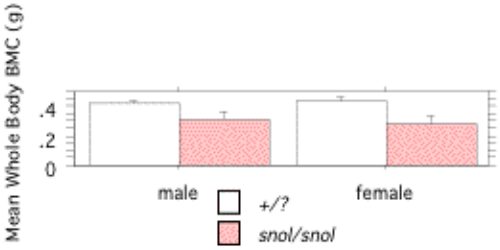
Skeletal and Craniofacial Morphology

Whole body bone mineral density (BMD) and bone mineral content (BMC) assessed by PIXImus densitometry (GE LUNAR, Madison, WI) were less in mutants than in controls, but differences were not statistically significant.

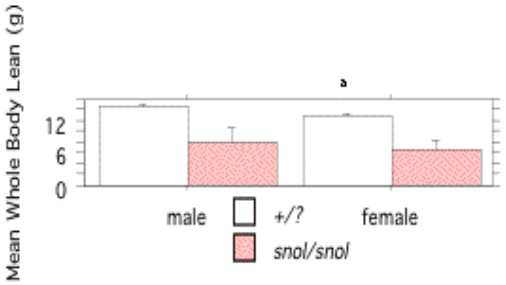
Graph 1: 8 Week Snubnose-like (*snol*)
Whole Body BMD (g/cm2)
Split by Sex and Genotype
(n=3; mean±SEM)



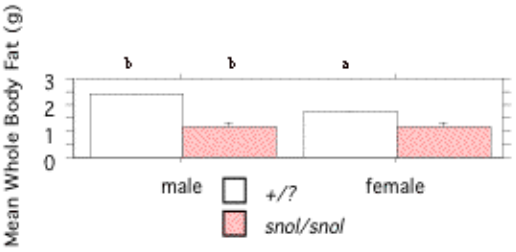
Graph 2: 8 Week Snubnose-like (*snol*)
Whole Body BMC (g)
Split by Sex and Genotype
(n=3; mean±SEM)



Graph 3: 8 Week Snubnose-like (*snol*)
Whole Body Lean (g)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)

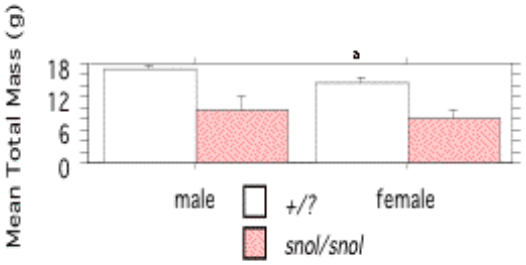


Graph 4: 8 Week Snubnose-like (*snol*)
Whole Body Fat (g)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃᵇp≤0.05)

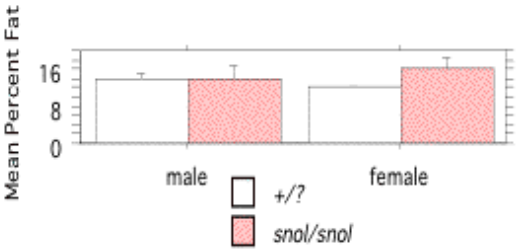


Skull BMC was significantly less in female mutants than female controls; it was less in male mutants than controls but not significant. Skull BMD had no statistical significance between the sexes even though mutants were less than controls. Total body mass and lean mass were significant with female controls greater than mutants. Male mutants were less than controls but not significant. Percent of fat was similar in male mutants and controls, while it was higher in female mutants than female controls.

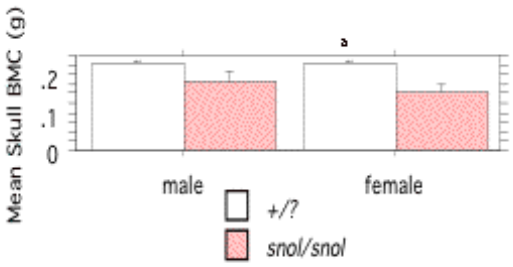
Graph 5: 8 Week Snubnose-like (*snol*)
Total Mass (g)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



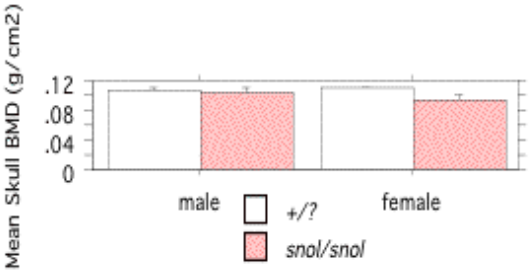
Graph 6: 8 Week Snubnose-like (*snol*)
Percent Fat
Split by Sex and Genotype
(n=3; mean±SEM)



Graph 7: 8 Week Snubnose-like (*snol*)
Skull BMC (g)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)

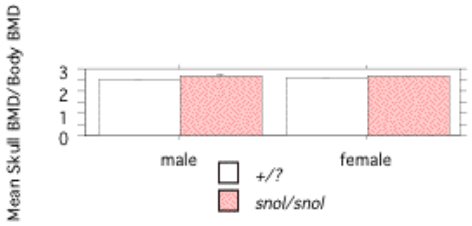


Graph 8: 8 Week Snubnose-like (*snol*)
Skull BMD (g/cm²)
Split by Sex and Genotype
(n=3; mean±SEM)

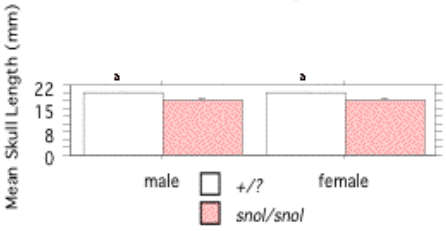


Morphological measurements of the skull were made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks. Skull height, skull width and inner canthal distance were equal or less in mutants compared to controls but not statistically different. However, skull length, nose length and lower and upper jaw lengths were all significantly shorter in female and male mutants. Jaw length ratio, skull to nose length ratio and skull height to length ratio were similar in mutants and controls. Two ratios were significant: skull length to width in male mutants was less than in controls, and skull height to width was less in female mutants than female controls.

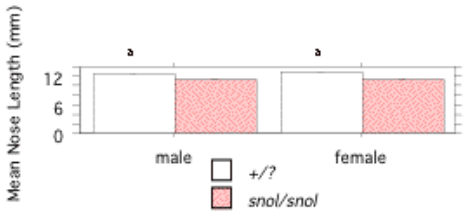
Graph 9: 8 Week Snubnose-like (*snol*)
Skull BMD/Body BMD
Split by Sex and Genotype
(n=3; mean±SEM)



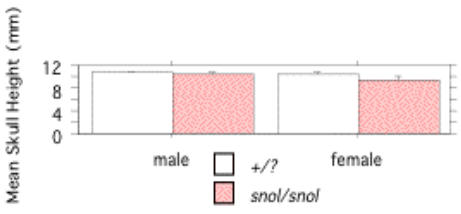
Graph 10: 8 Week Snubnose-like (*snol*)
Skull Length (mm)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



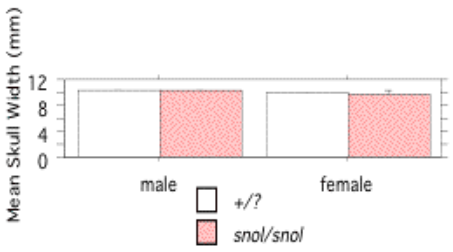
Graph 11: 8 Week Snubnose-like (*snol*)
Nose Length (mm)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



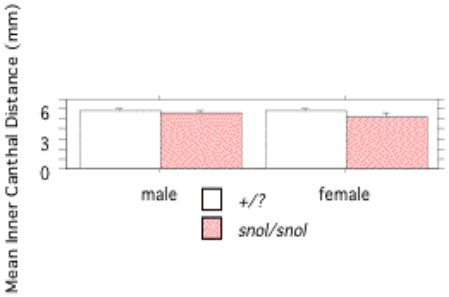
Graph 12: 8 Week Snubnose-like (*snol*)
Skull Height (mm)
Split by Sex and Genotype
(n=3; mean±SEM)



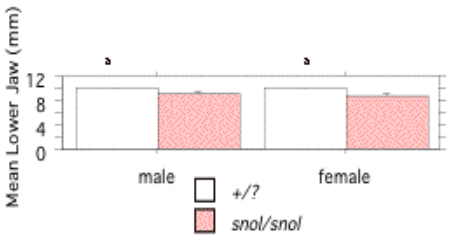
Graph 13: 8 Week Snubnose-like (*snol*)
Skull Width (mm)
Split by Sex and Genotype
(n=3; mean±SEM)



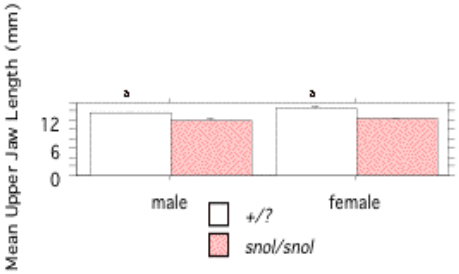
Graph 14: 8 Week Snubnose-like (*snol*)
Inner Canthal Distance (mm)
Split by Sex and Genotype
(n=3; mean±SEM)



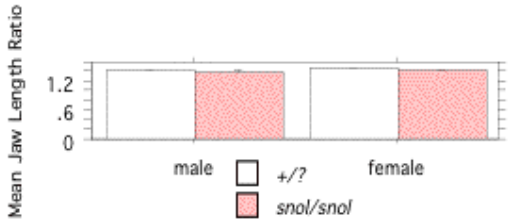
Graph 15: 8 Week Snubnose-like (*snol*)
Lower Jaw Length (mm)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



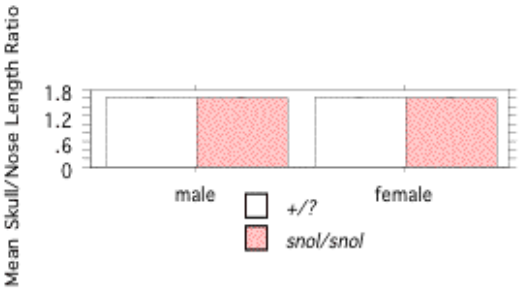
Graph 16: 8 Week Snubnose-like (*snol*)
Upper Jaw Length (mm)
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



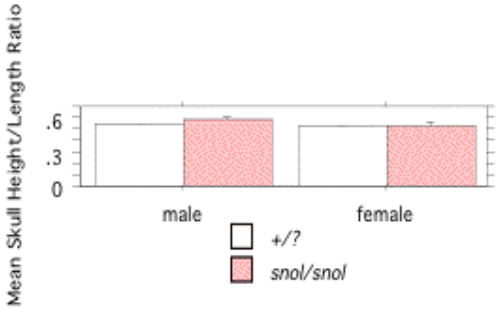
Graph 17: 8 Week Snubnose-like (*snol*)
Jaw Length Ratio
Split by Sex and Genotype
(n=3; mean±SEM)



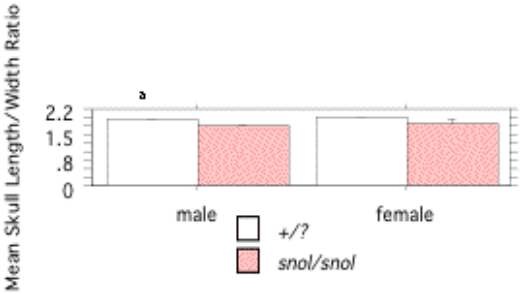
Graph 18: 8 Week Snubnose-like (*snol*)
Skull/Nose Length Ratio
Split by Sex and Genotype
(n=3; mean±SEM)



Graph 19: 8 Week Snubnose-like (*snol*)
Skull Height/Length Ratio
Split by Sex and Genotype
(n=3; mean±SEM)



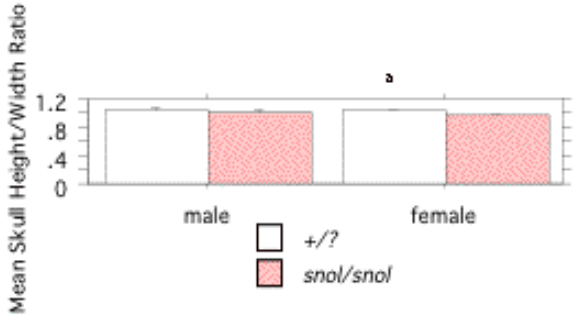
Graph 20: 8 Week Snubnose-like (*snol*)
Skull Length/Width Ratio
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



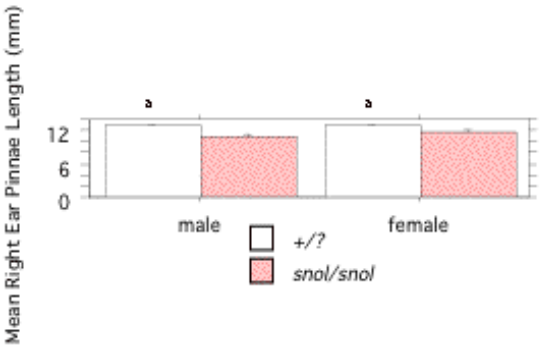
a +/? vs. nm/nm within sex
b female vs. male within sex

Mutant male and female mice had significantly shorter ear pinna than their controls. When comparing pinna length to skull height, the ratio was significantly less in male mutants than male controls and was significantly less in male mutants than to female mutants. Female controls and female mutants were the same.

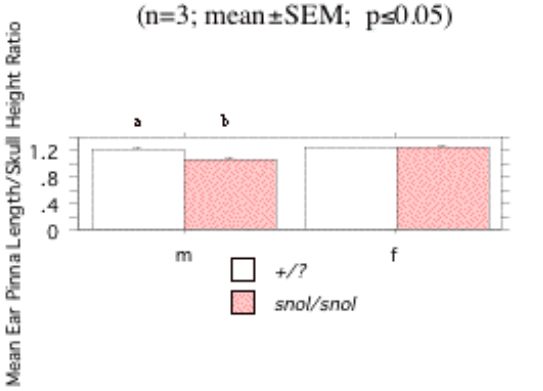
Graph 21: 8 Week Snubnose-like (*snol*)
Height/Width Ratio
Split by Sex and Genotype
(n=3; mean±SEM; ᵃp≤0.05)



Graph 22: 8 Week Snubnose-like (*snol*)
Right Ear Pinnae Length (mm)
Split by Sex and Genotype
(n=3; mean±SEM; [a]p≤0.05)



Graph 23: 8 Week Snubnose-like (*snol*)
Right Ear Pinna Length/Skull Height Ratio
Split by Sex and Genotype
(n=3; mean±SEM; p≤0.05)



a +/? vs. nm/nm within sex
b female vs. male within sex

Table 1: Digital Caliper Measurements and Calculated Ratios of Eight Week Old C3H/HeJ-nm2670 Skulls Stained with Alizarin Red (n=3; mean?SEM)

Measurements	Female +/? n=3	Female nm/nm n=3	Male +/?* n=3	Male nm/nm n=3
Whole body BMD (g/cm^2)	0.0427?0.00124	0.0353?0.00356	0.0428?0.00069	0.0391?0.00283
Skull BMD/body BMD	2.5584?0.06549	2.6471?0.06030	2.5098?0.01326	2.6608?0.07766
Whole body BMC (g)	0.432?0.0247	0.276?0.0622	0.415?0.0182	0.307?0.0498
Whole body lean (g)	12.8?0.64 [a]	6.7?1.75	14.6?0.59	8.1?2.72
Whole body fat (g)	1.7?0.03 [a b]	1.2?0.14 [b]	2.4?0.06 [a]	1.2?0.18
Total mass (g)	14.6?0.67 [a]	7.9?1.86	17.0?0.61	9.3?2.87
% fat	12	16	14	14
Skull BMD (g/cm^2)	0.1092?.00035	0.0931?0.00763	0.1075?0.00179	0.1037?0.00621
Skull BMC (g)	0.228?0.0061 [a]	0.156?0.0196	0.228?0.0089	0.183?0.0235

Table 2: PIXImus Densitometric Measurements of Eight Week Old C3H/HeJ-nm2670 Exvivo Skulls
(n=3; mean?SEM)

Table 2: PIXImus Densitometric Measurements of Eight Week Old C3H/HeJ-nm2670 Exvivo Skulls (n=3; mean?SEM)

Measurements	Female +/? n=3	Female nm/nm n=3	Male +/? n=3*	Male nm/nm n=3
Skull length (mm)	20.08?0.126 [a]	17.80?0.260	19.76?0.091 [a]	17.67?0.294
Nose length (mm)	12.71?0.076 [a]	11.30?0.079	12.45?0.062 [a]	11.14?0.127
Skull height (mm)	10.41?0.138	9.45?0.527	10.57?0.250	10.31?0.495
Skull width (mm)	10.04?0.116	9.79?0.509	10.24?0.129	10.17?0.197
Inner canthal distance (mm)	5.89?.178	5.27?0.290	5.85?0.115	5.68?0.101
Lower jaw length (mm)	9.98?0.139 [a]	8.64?0.215	9.90?0.094 [a]	8.89?0.288
Upper jaw length (mm)	14.65?0.289 [a]	12.24?0.306	13.93?0.063 [a]	12.18?0.165
Jaw length ratio	1.47?0.016	1.42?0.009	1.41?0.018	1.37?0.049
Skull/nose length ratio	1.58?0.003	1.58?0.019	1.59?0.002	1.59?0.031
Skull height/length ratio	0.52?0.005	0.53?0.027	0.54?0.015	0.58?0.018
Skull length/width ratio	2.00?0.014	1.83?0.087	1.93?0.025 [a]	1.74?0.011
Skull height/width ratio	1.04?0.003 [a]	0.96?0.006	1.03?0.021	1.01?0.029
Right Ear Pinna Length	12.85?0.153 [a]	11.59?0.422	12.80?0.142 [a]	10.86?0.500
Right Ear Pinna Length/ Skull Height Ratio	1.235?0.0131	1.230?0.0310 [b]	1.212?0.0370 [a]	1.054?0.0337

p?0.05 +/? vs. nm/nm within sex
b p?0.05 female vs. male within genotype
* 2 controls are +/snol

Acknowledgments

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References

Green,MC (1952) A rapid method for clearing and staining specimens for the demonstration of bone. The Ohio Journal of Science 52(1):31-33. January 1952.

Mouse Genome Database (MGD) Mouse Genome Informatics Project, The Jackson Laboratory, Bar, Harbor, Maine. World Wide Web (www.informatics.jax.org)

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, and Warman ML(2000) Preparation of PCR-Quality Mouse Genomic DNA with Hot Sodium Hydroxide and Tris (HotSHOT). Biotechniques 29:52-54

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4,303-313

Ward-Bailey PF, Johnson KR, Handel MA, Harris BH, Davisson MT. (1996) A new mouse mutation causing male sterility and histoincompatibility. Mamm. Genome 7, 793- 797.

Zheng QY, Johnson KR, Erway LC(1999) Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res 130, 94-107.